UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

OPP OFFICIAL RECORD
HEALTH EFFECTS DIVISION
SCIENTIFIC DATA REVIEWS
EPA SERIES 361

OFFICE OF PREVENTION, PESTICIDE AND TOXIC SUBSTANCES

MEMORANDUM

Date: July 22. 2008

SUBJECT: **2, 4-D**: Review of a 28-Day Subchronic Inhalation Toxicity Study in Rats *via* Nose Only Exposures.

PC Code: 030001
Decision No.: 392915
Petition No.: none
Risk Assessment Type: none
TXR No.: 0054870
MRID No.: 47398701

DP Barcode: D352172
Registration No.: RR-030001-27749
Regulatory Action: project
Case No.: none
CAS No.: 94-75-7
40 CFR: 180.142

FROM: Linda L. Taylor, Ph.D.
Reregistration Branch I
Health Effects Division (7509P)

THROUGH: Michael Metzger, Branch Chief
Reregistration Branch I
Health Effects Division (7509P)

TO: Tom Myers
Special Review and Reregistration Division (7508P)

I. **ACTION REQUESTED:** Review the 28-day subchronic inhalation study on 2, 4-D.

II. **CONCLUSIONS:** The 28-day subchronic inhalation study is classified acceptable and it satisfies the guideline (870.3465) requirement for a subchronic inhalation toxicity study.

III. **HED COMMENTS/DISCUSSION:** The Industry Task Force II on 2,4-D Research Data submitted a subchronic inhalation toxicity study in response to the Data Call-In for 2, 4-dichlorophenoxyacetic acid. The study has been reviewed, and the Data Evaluation Record is appended.

In a subchronic inhalation toxicity study (MRID 47398701), 2,4-D (99% a.i., Lot # 2006 24833 8006-USA) was administered to 10 or 20 Sprague-Dawley CD® rats/sex/

concentration by dynamic [nose only] exposure at concentrations of 0, 0.05, 0.10, 0.30 or 1.00 mg/L for 6 hours per day, 5 days/week for 28 days (for a total of at least 20 exposures).

Following nose-only inhalation exposure, 2, 4-D was associated with portal-of-entry effects that consisted of squamous metaplasia and epithelial hyperplasia with increased mixed inflammatory cells within the larynx. The incidence and severity of the effects were increased in a dose-related manner, and the effects persisted following the 4-week recovery period, although the incidence and severity were reduced. Clinical signs associated with exposure at the high dose included excessive salivation (day 13 and subsequently), labored breathing (day 13 and subsequently), and chromodacryorrhea (day 12 and intermittently thereafter). A slight decrease in body weight was observed in the high-dose females by day 14 and continued throughout the remainder of the dosing (↓10%) and the recovery periods (↓12%). Body-weight gains were reduced in the high-dose female group throughout the study and recovery period and were accompanied by a reduction in food intake. Although a treatment-related reduction in reticulocyte counts was observed at the mid-high and high-dose levels in both sexes at terminal sacrifice, the toxicological significance is not known. Alkaline phosphatase values were increased in the mid-high and high-dose females and aspartate aminotransferase values were increased slightly in the high-dose females, but no correlating microscopic pathology findings were observed. Lung weights were unaffected by treatment. Females at the high-dose level displayed slight reductions in spleen and thymus weights. Organ weights were comparable among the male groups.

The systemic toxicity LOAEL is 1.0 mg/L/day, based on increased alkaline phosphatase and aspartate aminotransferase levels in females and decreased spleen weights in females. The NOAEL is 0.30 mg/L/day.

A NOAEL for portal-of-entry effects was not determined. The LOAEL for portal-of-entry effects (squamous metaplasia and epithelial hyperplasia with increased mixed inflammatory cells within the larynx; not totally resolved following a 4-week recovery period) **is 0.05 mg/L, the lowest dose tested.**

This subchronic inhalation toxicity study in the rat is acceptable (guideline), and it satisfies the guideline requirement for a subchronic inhalation study (OPPTS 870.3465; OECD 413) in the rat.

EPA Reviewer: Linda Taylor, Ph.D. **Signature:**
Reregistration Branch I, Health Effects Division (7509C) **Date:** 07-16-08
EPA Secondary Reviewer: Kit Farwell, DVM **Signature:**
Reregistration Branch I, Health Effects Division (7509C) **Date:** 7-16-08

Template version 02/06

TXR# 0054870

DATA EVALUATION RECORD

STUDY TYPE: Subchronic Inhalation Toxicity - rat; OPPTS 870.3465 [§82-4]; OECD 413.

PC CODE: 030001 **DP BARCODE:** D352172

TEST MATERIAL (PURITY): 2,4-Dichlorophenoxyacetic acid (2,4-D; 99% a.i.)

SYNONYMS: Industry Task Force II on 2,4-D Research Data

CITATION: Hoffman, G. M. (2008). A 28-Day Subchronic Inhalation Toxicity Study of 2,4-Dichlorophenoxyacetic Acid in the Rat *via* Nose-Only Exposures. Huntingdon Life Sciences, NJ. Study Number 07-6156, July 19, 2007-March 26, 2008. MRID 47398701. Unpublished.

SPONSOR: Industry Task Force II on 2, 4-D Research Data

EXECUTIVE SUMMARY: In a subchronic inhalation toxicity study (MRID 47398701), 2,4-D *(99% a.i., Lot # 2006 24833 8006-USA)* was administered to 10 or 20 Sprague-Dawley CD® rats/sex/concentration by dynamic [nose only] exposure at concentrations of 0, 0.05, 0.10, 0.30 or 1.00 mg/L for 6 hours per day, 5 days/week for 28 days (for a total of at least 20 exposures).

Following nose-only inhalation exposure, 2, 4-D was associated with portal-of-entry effects that consisted of squamous metaplasia and epithelial hyperplasia with increased mixed inflammatory cells within the larynx. The incidence and severity of the effects were increased in a dose-related manner, and the effects persisted following the 4-week recovery period, although the incidence and severity were reduced. Clinical signs associated with exposure at the high dose included excessive salivation (day 13 and subsequently), labored breathing (day 13 and subsequently), and chromodacryorrhea (day 12 and intermittently thereafter). A slight decrease in body weight was observed in the high-dose females by day 14 and continued throughout the remainder of the dosing (↓10%) and the recovery periods (↓12%). Body-weight gains were reduced in the high-dose female group throughout the study and recovery period and were accompanied by a reduction in food intake. Although a treatment-related reduction in reticulocyte counts was observed at the mid-high and high-dose levels in both sexes at terminal sacrifice, the toxicological significance is not known. Alkaline phosphatase values were increased in the mid-high and high-dose females and aspartate aminotransferase values were increased slightly in the high-dose females, but no correlating microscopic pathology findings were observed. Lung weights were unaffected by treatment. Females at the high-dose level displayed slight reductions in spleen and thymus weights. Organ weights were comparable among the male groups.

The systemic toxicity LOAEL is 1.0 mg/L/day, based on increased alkaline phosphatase and aspartate aminotransferase levels in females and decreased spleen weights in females. The NOAEL is 0.30 mg/L/day.

A NOAEL for portal-of-entry effects was not determined. The LOAEL for portal-of-entry effects (squamous metaplasia and epithelial hyperplasia with increased mixed inflammatory cells within the larynx; not totally resolved following a 4-week recovery period) **is 0.05 mg/L, the lowest dose tested.**

This subchronic inhalation toxicity study in the rat is acceptable (guideline), and it satisfies the guideline requirement for a subchronic inhalation study (OPPTS 870.3465; OECD 413) in the rat.

COMPLIANCE: Signed and dated GLP, Quality Assurance, and Data Confidentiality statements were provided.

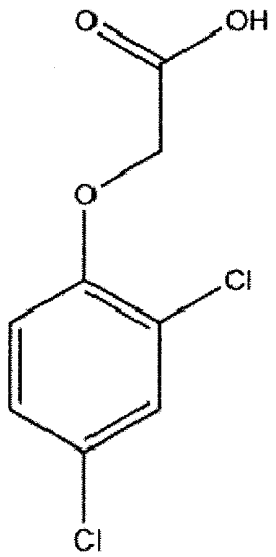
I. MATERIALS AND METHODS:

A. MATERIALS:

1. Test material: 2,4-Dichlorophenoxyacetic acid

Description:	white powder
Lot/batch #:	Lot # 2006 24833 8006-USA)
Purity:	99% a.i.
Compound stability:	expiration date 1/9/2009
CAS # of TGAI:	94-75-7
Structure:	

Molecular Structure:



2. Vehicle and/or positive control: N/A (administered as a dust)

3. Test animals:

Species:	rat	
Strain:	Spregue Dawley CD®; Crl:CD (SD) IGS BR Vaf/Plus®	
Age/weight at study initiation:	males 8 weeks (mean 276; range 223-334 g) females 9 weeks (mean 212; range 187-234 g)	
Source:	Charles River Laboratories, Raleigh, NC	
Housing:	individually housed (suspended, stainless steel wire mesh cages)	
Diet:	Certified Rodent Diet, No. 2016C pellets *ad libitum* (except during exposure)	
Water:	Municipal, *ad libitum* (except during exposures)	
Environmental conditions:	**Temperature:**	20-21EC
	Humidity:	53-66%
	Air changes:	#/hr (not provided)
	Photoperiod:	12 hrs dark/12 hrs light
Acclimation period:		

B. STUDY DESIGN:

1. In life dates: Start: July 19, 2007; End: March 26, 2008.

2. Animal assignment: Animals were assigned randomly (computerized random sort program;

group body-weight means comparable) and distributed into 3 groups of 10 rats per sex and 2 groups of 20 rats per sex (Table 1).

Table 1. Study Design

Group	Exposure level (mg/L)	Initial M/F	Clinical lab studies		Necropsy		Pathology	Nominal concentration (mg/L)	Gravimetric concentration (mg/L)	MMAD (μm)	GSD
			main	recovery	main	recovery					
Control	0.00	20/20	10/10	10/10	10/10	10/10	20/20	-	0.00	-	-
Low	0.05	10/10	10/10	0/0	10/10	0/0	10/10	0.062	0.048	1.7	1.98
mid-1	0.10	10/10	10/10	0/0	10/10	0/0	10/10	0.19	0.11	2.1	1.87
mid-2	0.30	10/10	10/10	0/0	10/10	0/0	10/10	0.59	0.34	2.1	1.95
High	1.00	20/20	10/10	10/10	10/10	10/10	20/20	1.9	1.00	2.6	1.89

From pages 13, 34, and 35

3. **Dose selection rationale:** The dose levels were selected based on the results from an acute inhalation study (HLS Study No. 86-7893), which showed limited effects (no mortality but transient decreased activity, salivation, lacrimation, nasal discharge, and labored breathing during and after exposure) at 1.79 mg/L.

4. **Generation of the test atmosphere / chamber description:** The nose-only chamber had a volume of 50 L and was operated dynamically under slight positive pressure. This chamber size and airflow rate (airflow rate maintained at a total flow rate of 22 Lpm) were considered adequate to maintain the rat loading factor below 5% and oxygen above 19%. After being sieved through a stainless steel sieve, the test material was packed into a dust feeder cup using a press. The cup was then mounted onto the dust feeder.

   Pre-study chamber distribution analyses showed the test material was evenly distributed within each chamber. The **achieved mean exposure concentration** of each group was very close to the target concentration (Table 1). The differences between measured and nominal concentrations were said to be typical of this type of exposure and considered representative of test material depositing within the exposure chambers (Table 1). The results of the **particle size distribution** measurements indicated that the aerosols for all test material exposures were similar in size. Measurements during the air control exposures showed that the air in that chamber was comparable to room air.

5. **Statistics:** Statistical analyses were performed on: body weight/body-weight change, feed consumption, hematology, coagulation, clinical chemistry, organ weight data. Multiple Group Analysis: Bartlett's test was performed to determine if groups had equal variances. If variances were equal, parametric procedures were used; if not, nonparametric procedures were used (body weight and organ weight data were analyzed only by parametric methods). The parametric method was the standard one-way analysis of variance (ANOVA) using the F ratio to assess significance (Armitage). If significant differences among the means were indicated, additional tests were used to determine which means were significantly different from the control (Dunnent's, Williams, or Cochran and Cox's modified t-test. The nonparametric method was the Kruskal-Wallis test and if differences were indicated, Shirley's test or Steel' test were used to determine which means differed from control. Bartlett's test for equality of variance was conducted at the 1% significance level; all other statistical tests were conducted and the 5% and 1% significance levels. Two Group Analysis: The parametric method was the standard one-way analysis of variance (ANOVA) using the F ratio to assess significance (Armitage) to determine if

treated group mean differed from control. The nonparametric method was the Kruskal-Wallis test followed by Wilcoxon's test to determine if treated group means differed from control.

C. METHODS:

1. Observations:

1a. Cageside observations: Animals were inspected twice daily for signs of toxicity and mortality. During each exposure, rats were observed as a group at least once.

1b. Clinical examinations: Each rat was removed from its cage and examined once pretest and once weekly (post-exposure) during the study period. Examinations included observations of general condition, skin and fur, eyes, nose, oral cavity, abdomen and external genitalia; and the occurrence of secretions and excretions and autonomic activity (lacrimation, piloerection, pupil size, and unusual respiratory pattern) were recorded

1c. Neurological evaluations: Although no specific neurological examination was performed, as part of the clinical examination, changes in gait, posture, and response to handling, as well as the presence of clonic or tonic movements, stereotypy (excessive grooming, repetitive circling) or bizarre behavior (self-mutilation, walking backward) were recorded.

2. **Body weight:** Animals were weighed twice pretest and weekly throughout the study. Terminal (fasted) weights were obtained just prior to necropsy.

3. **Food consumption:** Feed was available without restriction 7 days/week. Full feeders of known weight were presented and after 6 days, the feeders were reweighed and the resulting weight was subtracted from the full-feeder weight to obtain the amount consumed per rat over the 6-day period. Food consumption for each animal was determined once pretest and once a week during the treatment and recovery periods. Mean daily diet consumption was calculated as g food/kg body weight/day. Food efficiency was not evaluated.

4. **Ophthalmoscopic examination:** Eyes were examined pretest and at study termination. Lids, lacrimal apparatus and conjunctiva were examined visually. The cornea, anterior chamber, lens, iris, vitreous humor, retina, and optic disc were examined by indirect ophtholmoscopy. The eyes were examined after instillation of a mydriatic (Tropicamide Ophthalmic Solution, 1%).

5. **Hematology and clinical chemistry:** Blood was obtained *via* puncture of the vena cava under isoflurane anesthesia (rats fasted overnight) for analyses of hematology (tubes containing EDTA anticoagulant), coagulation (tubes containing sodium citrate anticoagulant), and clinical chemistry parameters for up to 10 rats/sex from the control and high-dose groups at study termination and recovery. The CHECKED (X) parameters were examined.

a. Hematology:

X	Hematocrit (HCT)*	X	Leukocyte differential count*
X	Hemoglobin (HGB)*	X	Mean corpuscular HGB (MCH)*
X	Leukocyte count (WBC)*	X	Mean corpusc. HGB conc.(MCHC)*
X	Erythrocyte count (RBC)*	X	Mean corpusc. volume (MCV)*
X	Platelet count*	X	Reticulocyte count
X	Blood clotting measurements*	X	Red cell distribution width
X	(Activated partial thromboplastin time)	X	Mean platelet volume
	(Clotting time)		
X	(Prothrombin time)		

* Recommended for subchronic inhalation studies based on Guideline 870.3465

b. Clinical chemistry:

X	ELECTROLYTES	X	OTHER
X	Calcium	X	Albumin*
X	Chloride	X	Creatinine*
	Magnesium	X	Urea nitrogen*
X	Phosphorus	X	Total Cholesterol*
X	Potassium*	X	Globulins
X	Sodium*	X	Glucose*
X	**ENZYMES**	X	Total bilirubin
X	Alkaline phosphatase*	X	Total serum protein (TP)*
	Cholinesterase (ChE)	X	Triglycerides
	Creatine phosphokinase		Serum protein electrophoresis
	Lactic acid dehydrogenase (LDH)		
X	Alanine aminotransferase (ALT/also SGPT)*		
X	Aspartate aminotransferase (AST/also SGOT)*		
	Sorbitol dehydrogenase*		
X	Gamma glutamyl transferase (GGT)*		
	Glutamate dehydrogenase		

* Recommended for subchronic inhalation studies based on Guideline 870.3465

6. **Urinalysis**: Urine was not collected.

7. **Sacrifice and pathology:** Necropsy was performed on 10 rats/sex/group after the 4-week exposure period and on the 10 rats/sex/per recovery group (control and high dose) after the 4-week recovery period. All animals were fasted overnight prior to sacrifice (exsanguination following isoflurane inhalation). The macroscopic examination included of external surfaces and all orifices; external surfaces of the brain and spinal cord; the organs and tissues of the cranial, thoracic, abdominal and pelvic cavities and neck; and the remainder of the carcass. The CHECKED (X) tissues were collected for histological examination. The (XX) organs, in addition, were weighed.

X	DIGESTIVE SYSTEM	X	CARDIOVASC./HEMAT.	X	NEUROLOGIC
	Tongue	X	Aorta, thoracic*	XX	Brain*+
X	Salivary glands*	XX	Heart*+	X	Peripheral nerve*
X	Esophagus*	X	Bone marrow*	X	Spinal cord (3 levels)*
X	Stomach*	X	Lymph nodes*	X	Pituitary*
X	Duodenum*	XX	Spleen*+	X	Eyes (optic nerve)*
X	Jejunum*	XX	Thymus*+	X	GLANDULAR
X	Ileum*			XX	Adrenal gland*+
X	Cecum*	X	UROGENITAL	X	Lacrimal gland
X	Colon*	XX	Kidneys*+	X	Parathyroid*
X	Rectum*	X	Urinary bladder*	X	Thyroid*
XX	Liver*+	XX	Testes*+	X	OTHER
	Gall bladder* (not rat)	XX	Epididymides*+	X	Bone (sternum and/or femur)
	Bile duct* (rat)	X	Prostate*	X	Skeletal muscle
X	Pancreas*	X	Seminal vesicles*	X	Skin
X	RESPIRATORY	XX	Ovaries*+	X	All gross lesions and masses*
X	Trachea*	XX	Uterus*+	X	Zymbal's gland
XX	Lung*+	X	Mammary gland*		
X	Nose*				
	Pharynx*				
X	Larynx*				

* Recommended for subchronic rodent studies based on Guideline 870.3465
+ Organ weights required

II. RESULTS:

A. OBSERVATIONS :

1. **Clinical signs of toxicity:** Excessive salivation, labored breathing, and chromodacryorrhea were observed in several of the rats at the high-dose level following the 12th exposure and continuing during the remainder of the exposures. Wet fur was observed more frequently in the treated rats than the control.

2. **Mortality:** All rats survived until the scheduled sacrifice.

3. **Neurological evaluations:** No specific neurological assessments were performed, other than general observation of behavior and movement during the routine clinical signs assessment.

B. BODY WEIGHT AND WEIGHT GAIN: Body weight and body-weight gain were comparable among the males throughout the exposure period. At the beginning of the recovery period, the 10 male rats in the high-dose group displayed a statistically-significant reduction in body weight compared to the recovery control rats (90% of control). The body-weight gain observed for the period between the last exposure day and day 7 of the recovery period was reduced in the high-dose male group compared to the control (35.2 grams vs 13.1 grams). This is not considered to be a treatment-related finding. Females at the high dose level displayed a slight decrease in body weight by day 14 compared to the control, which continued throughout the remainder of the dosing period (↓10%) and the recovery period (↓12%). Body-weight gain was reduced throughout the exposure and recovery periods in the high-dose female group (Tables 2 and 3).

Table 2. Body-Weight Data (grams)					
mg/L	0	0.05	0.10	0.30	1.00
Males					
Pre-test (day 14)	228±12	276±12	273±20	276±12	278±16
Day 7	294±16	291±18	288±23	290±18	294±22
Day28	352±27	348±35	340±36	342±27	336±26
Recovery					
Day 7	386±26	-	-	-	348±24** (90)
Day28	470±32	-	-	-	458±28
Females					
Pre-test (day 14)	212±12	212±13	214±10	212±12	210±10
Day 7	223±12	222±16	222±14	220±12	214±14
Day 14	228±11	227±19	226±18	226±14	212±16** (92)
Day 21	239±12	238±20	230±20	234±16	218±19** (90)
Day 28	250±18	242±23	236±22	240±16	225±20** (90)
Recovery					
Day 7	264±18	-	-	-	231±20** (88)
Day 14	275±20	-	-	-	244±26** (89)
Day 21	285±25	-	-	-	251±27** (88)
Day 28	298±25	-	-	-	260±28** (88)

Data from Table 4, pages 65-66 of the report; - not weighed; ** $p<0.01$; (% of control)

Table 3. Body-Weight Gain Data (grams)					
mg/L	0	0.05	0.10	0.30	1.00
Males					
Exposure					
Day 7	17±7	14±14	15±8	14±10	16±8
Day28	74±21	71±32	66±25	65±22	58±14
Recovery					
Day 7	107±20	-	-	-	76±21** (70)
Day14	136±20	-	-	-	114±20* (84)
Day 28	190±22	-	-	-	184±23
Females					
Exposure					
Day 7	11±7	10±6	8±6	8±4	4±6** (32)
Day 14	16±11	15±10	12±10	14±10	2±10** (14)
Day 21	28±13	26±12	16±12	22±11	6±14** (24)
Day 28	38±16	30±14	22±14*	28±13	14±15** (38)
Recovery					
Day 7	48±18	-	-	-	20±13** (43)
Day 14	60±22	-	-	-	34±16** (58)
Day 21	70±22	-	-	-	41±18** (59)
Day 28	82±22	-	-	-	50±19** (62)

Data from Table 5, pages 67-68; # provided in report are change from baseline; - not weighed; ** $p<0.01$; (% of control)

C. **FOOD CONSUMPTION:** Food consumption was comparable among the male groups throughout the study. Females displayed a slight reduction (~10%) in food consumption compared to the control group throughout the exposure and recovery periods.

D. **OPHTHALMOSCOPIC EXAMINATION:** No alterations were observed in either sex.

E. **BLOOD ANALYSES:**

1. **Hematology:** There was a treatment-related reduction in reticulocyte counts that attained

statistical significance at the mid-high and high-dose levels (magnitude of response similar at both dose levels) in both sexes (terminal sacrifice). The reduction showed reversibility in the males but persisted in the high-dose females after the 4-week recovery period. There were no correlating microscopic pathology findings in either sex. Other findings in females included reductions in WBC (high) and absolute neutrophils (mid-high and high) and lymphocytes (high). Since none of these were observed in the male groups, pre-exposure values were not provided (reduction remain following recovery), and there are no correlating microscopic pathology, the findings are not considered toxicologically significant (Table 4).

Table 4. Hematology Parameters

mg/L	0	0.05	0.10	0.30	1.00
Males					
Reticulocyte ($x10^{-6}$/µL)	216.7±35.97	234.2±76.98	178.0±21.15	160.6±41.85** (74)	159.4±27.45** (74)
4-week recovery	271.1±33.63	-	-	-	309.5±36.85* (114)
Females					
Reticulocyte ($x10^{-6}$/µL)	193.7±35.79	176.1±33.52	174.9±34.48	154.0±33.22* (80)	153.0±34.24* (79)
4-week recovery	204.5±55.38	-	-	-	164.1±18.44 (80)
WBC ($x10^{-3}$/µL)	11.30±4.088	9.18±2.043	10.21±2.658	9.41±3.908	7.72±2.763* (68)
4-week recovery	9.04±2.891	-	-	-	7.52±2.994 (83)
Ab neutrophil ($x10^{-3}$/µL)	1.43±1.049	0.85±0.162	0.92±0.327	0.83±0.467* (58)	0.80±0.445* (56)
4-week recovery	1.00±0.334	-	-	-	0.73±0.261 (73)
Ab lymphocyte ($x10^{-3}$/µL)	9.41±3.183	8.00±2.010	8.95±2.445	8.20±3.475	6.65±2.611* (70)
4-week recovery	7.57±2.633	-	-	-	6.51±2.772 (86)

Data from Table 7 (Appendix I), pages 72-79 of the report; * p<0.05; ** p<0.01; (% of control)

2. **Clinical chemistry:** High-dose females displayed slight, reversible, treatment-related changes that included increased serum alkaline phosphatase (40%) and increased aspartate aminotransferase (35%) compared to control values. The mid-high females also displayed a statistically-significant increase in alkaline phosphatase (124). The high-dose males also displayed an increased ALT value compared to the control; however, the apparent increase is attributed to one value (136), which was nearly 4 times greater than the other values (standard deviation >30). There were no correlating histopathological findings in the liver in either sex (Table 5).

Table 5. Clinical Chemistry Parameters

mg/L	0	0.05	0.10	0.30	1.00
	Males				
ALT (UL)	36±5.2	33±6.1	37±6.2	37±6.1	52±30.7 (144)[A]
4-week recovery	39±7	-	-	-	36±6
	Females				
Alkaline phosphatase (UL)	77±16.4	88±23.3	83±16.0	96±20.6* (124)	108±18.9** (140)
4-week recovery	42±9.8	-	-	-	51±11.3
AST (UL)	124±36.1	116±32.7	139±14.8	136±30.5	167±52.1** (135)
4-week recovery	147±40.9	-	-	-	149±67.3
ALT (UL)	39±24.7	33±7.3	38±11.1	43±18.3	45±13.1 (115)
4-week recovery	36±16.9	-	-	-	48±31.6 (133)

Data from Table 9 (Appendix I), pages 84-91 of the report; * p<0.05; ** p<0.01; (% of control); [A] without one value {136}, mean 42±8.8 (116)

3. **Coagulation:** There were no differences in coagulation values in either sex.

F. **URINALYSIS:** not performed

G. SACRIFICE AND PATHOLOGY:

1. **Organ weight:** No treatment-related changes in lung weight were observed in either sex (Table 6). Females at the high-dose level displayed a slight, statistically-significant, reduction in spleen weight (absolute and relative to brain) compared to the control.

Table 6. Organ Weights

mg/L	0	0.05	0.10	0.30	1.00
Males					
Lung					
Absolute (g)	1.33±0.128	1.28±0.10	1.30±0.12	1.32±0.13	1.33±0.12
% BW	0.414±0.030	0.402±0.02	0.413±0.02	0.417±0.02	0.416±0.04
% brain wt.	65.72±6.32	65.28±5.58	64.46±4.76	64.16±4.91	66.14±5.48
Thymus					
Absolute (g)	0.38±0.09	0.39±0.08	0.30±0.06	0.34±0.15	0.34±0.0.10
% BW	0.117±-.023	0.121±0.020	0.097±0.017	0.104±0.042	0.108±0.024
% brain wt.	18.74±4.60	19.66±3.49	15.17±3.12	16.27±6.94	17.32±4.52
Spleen					
Absolute (g)	0.60±0.10	0.60±0.10	0.52±0.06	0.56±0.11	0.54±0.07
% BW	0.18±0.02	0.18±0.02	0.16±0.02	0.18±0.02	0.17±0.02
% brain wt.	29.76±4.82	30.62±4.45	26.16±2.14	27.20±4.94	27.24±2.84
Terminal BW	323±30	321±36	315±34	316±27	320±24
Recovery					
Spleen					
Absolute (g)	0.74±0.12				0.85±0.11* (116)
% BW	0.16±0.02				0.20±0.02** (118)
% brain wt	34.62±5.49				41.46±4.94** (120)
Terminal BW	440±30				428±26
Females					
Lung					
Absolute (g)	1.11±0.08	1.11±0.12	1.09±0.12	1.16±0.12	1.09±0.13
% BW	0.50±0.04	0.49±0.02	0.51±0.02	0.52±0.02	0.52±0.04
% brain wt.	60.08±4.63	59.90±5.71	58.96±5.77	61.80±5.39	58.46±5.50
Thymus					
Absolute (g)	0.38±0.07	0.40±0.11	0.40±0.09	0.35±0.06	0.34±0.10 (88)
% BW	0.17±0.03	0.17±0.04	0.18±0.04	0.16±0.02	0.16±0.04
% brain wt.	20.73±3.56	21.38±5.89	21.54±5.07	18.63±3.29	18.11±4.71 (87)
Spleen					
Absolute (g)	0.50±0.07	0.51±0.13	0.45±0.05	0.47±0.08	0.42±0.06* (85)
% BW	0.22±0.03	0.22±0.04	0.21±0.02	0.21±0.02	0.20±0.02
% brain wt.	26.96±3.94	27.68±7.16	24.29±2.56	25.12±4.70	22.79±2.98* (84)
Terminal BW	225±15	226±22	215±20	222±18	209±19 (92)
Recovery					
Thymus					
Absolute (g)	0.36±0.05				0.32±0.08 (88)
% BW	0.12±0.02				0.13±0.02
% brain wt	18.04±3.14				17.27±4.14 (96)
Spleen					
Absolute (g)	0.54±0.08				0.46±0.08* (84)
% BW	0.19±0.02				0.18±0.02
% brain wt	27.30±2.82				25.20±3.86 (92)
Lung					
Absolute (g)	1.26±0.12				1.12±0.09* (89)
% BW	0.45±0.04				0.46±0.02
% brain wt	63.61±5.76				61.57±4.17
Terminal BW	278±23				244±25 (87)

Data from Table 10 (Appendix I), pages 93-108 of the report; n=10; * $p<0.05$; ** $p<0.01$; (% of control)

2. **Gross pathology:** No treatment-related findings were reported in either sex.

3. **Microscopic pathology:** Exposure Phase: In the larynx of both sexes, microscopic findings were observed in all treatment groups (Table 6). These consisted of squamous/squamoid epithelial metaplasia with hyperkeratosis, hyperplasia of the arytenoids epithelium and increased numbers of mixed inflammatory cells. Minimal to moderate squamous/squamoid metaplaswas present in both sexes and all rats exposed to 2,4-D, with the severity of the lesions showing a dose-related increase. Recovery Phase: Squamous/squamoid metaplasia, hyperkeratosis, and arytenoids epithelial hyperplasia persisted in the larynx following the 4-week recovery period (Table 7), although the incidence and severity were reduced. A single area of minimal epithelial hyperplasia (focal, unilateral) occurred in the nasolacrimal duct of one female in the high-dose main study exposure group.

Table 6. Microscopic Changes in the Larnyx (Exposure Phase)

	Males					Females				
2,4-D (mg/L)	**0.00**	**0.05**	**0.10**	**0.03**	**1.00**	**0.00**	**0.05**	**0.10**	**0.03**	**1.00**
# larynx examined	10	10	10	10	9	10	10	10	10	10
V-SM-GVentral Epithelium Squamous/Squamoid Metaplasia										
Minimal	0	0	0	0	0	0	1	1	0	2
Slight	0	10	10	8	3	0	9	9	9	5
Moderate	0	0	0	2	6	0	0	0	1	3
Total incidence	0	10	10	10	9	0	10	10	10	10
V-SM-G Ventral Epithelium Hyperkeratosis										
Minimal	0	8	10	7	5	0	9	9	8	5
Slight	0	0	0	2	2	0	0	0	1	5
Moderate	0	0	0	1	1	0	0	0	0	0
Total incidence	0	8	10	10	8	0	9	9	9	10
V-SM-G Arytenoid Epithelium Hyperplasia										
Minimal	0	0	3	1	3	0	0	3	3	5
Slight	0	0	0	2	1	0	0	0	1	1
Moderate	0	0	0	1	2	0	0	0	0	0
Total incidence	0	0	3	4	6	0	0	3	4	6
V-SM-G Mixed Inflammatory Cells										
Minimal	5	1	1	1	0	6	2	1	2	2
Slight	4	9	4	4	1	4	7	6	5	6
Moderate	0	0	5	5	8	0	1	3	3	2
Total incidence	9	10	10	10	9	10	10	10	10	10

Data from Table V, page 40 of the report; V-SM-G Ventral Seromucinous Glands

Table 7. Microscopic Changes in the Larnyx (Recovery Phase)

	Males		Females	
2,4-D (mg/L)	**0.00**	**1.00**	**0.00**	**1.00**
# larynx examined	5	9	8	7
V-SM-GVentral Epithelium Squamous/Squamoid Metaplasia				
Minimal	0	5	1	2
Slight	0	1	0	0
Total incidence	0	6 (67)	1 (12)	2 (29)
V-SM-G Ventral Epithelium Hyperkeratosis				
Minimal	0	1	0	0
Total incidence	0	1 (11)	0	0
V-SM-G Arytenoid Epithelium Hyperplasia				
Minimal	0	0	0	1

Table 7. Microscopic Changes in the Larnyx (Recovery Phase)				
	Males		Females	
2,4-D (mg/L)	**0.00**	**1.00**	**0.00**	**1.00**
Total incidence	0	0	0	1
V-SM-G Mixed Inflammatory Cells				
Minimal	3	2	6	5
Slight	1	6	0	2
Total incidence	4 (80)	8 (89)	6 (75)	7 (100)

Data from Tables VI and VII, page 41 of the report; (%); V-SM-G Ventral Seromucinous Glands

III. DISCUSSION AND CONCLUSIONS

A. INVESTIGATORS' CONCLUSIONS: The 28-days of subchronic nose-only inhalation exposure to 2,4-dichlorophenoxyacetic acid in rats at 0.05, 0.10, 0.30, and 1.00 mg/L was associated with minimal to slight decreases in body weight gain, slight decreases in feed consumption, minimal, transient clinical signs during exposure, minimal to slight, generally reversible, alterations in clinical pathology parameters and squamous metaplasia and epithelial hyperplasia with increased mixed inflammatory cells within the larynx, with recovery, in the 1.00 mg/L exposed animals. The laryngeal findings were also noted with exposure level-related severity at the lower exposure levels. Laryngeal squamous metaplasia is considered an adaptive non-specific response to chronic irritation by which a susceptible epithelium is replaced by a more resistant one. Such induced changes usually fail to result in the development of lesions in primates (Lewis, 1991) and are not considered indicative of significant risk in humans (Osimitz, *et al.*, 2007). Therefore, a no observed adverse effect level (NOAEL) for systemic toxicity was determined to be 0.30 mg/L but a NOAEL for portal-of-entry toxicity was not determined for nose-only inhalation exposure in rats to 2, 4-dichlorphenoxyacetic acid.

B. REVIEWER COMMENTS: Following nose-only inhalation exposure, 2, 4-D was associated with portal-of-entry effects that consisted of squamous metaplasia and epithelial hyperplasia with increased mixed inflammatory cells within the larynx. The incidence and severity of the effects were increased in a dose-related manner. Clinical signs associated with exposure at the high dose included excessive salivation, labored breathing, and chromodacryorrhea. Females at the high dose level displayed a slight decrease in body weight by day 14, which continued throughout the remainder of the dosing (↓10%) and the recovery periods (↓12%). Body-weight gains were reduced in the high-dose female group throughout the study and recovery period. Other findings that were associated with treatment included a treatment-related reduction in reticulocyte counts that attained statistical significance at the mid-high and high-dose levels in both sexes (terminal sacrifice). The reduction showed reversibility in the males but persisted in the high-dose females after the 4-week recovery period. There were no correlating microscopic pathology findings in either sex, and the toxicological significance is not known. Alkaline phosphatase values were increased in the mid-high and high-dose females at study termination, but no correlating microscopic pathology findings were observed. Females at the high-dose level displayed slight reductions in spleen and thymus weights. Organ weights were comparable among the male groups.

C. STUDY DEFICIENCIES: Based on the fact that the microscopic changes in the larynx were observed in all dose groups, the lack of recovery groups for the lower dose levels is unfortunate, but this does not adversely affect study interpretation.